

Guidelines for Submitting Specimens for Influenza H5N1 Analysis Vermont Department of Health

Highly pathogenic avian influenza (H5N1) is classified as a select agent and the Centers for Disease Control and Prevention (CDC) has recommended that rapid and PCR assays (rather than culture) be performed from respiratory specimens on patients suspected of being infected with this agent or those who have traveled to a country with documented H5N1 avian influenza.

In order to assure the safety of our Laboratorians, the VDH Laboratory **is requiring a travel history (of the past 6 months) be obtained from all patients, and the VDHL-Clinical Test Request form be completed to reflect any travel when requesting an Influenza test.** The VDH Laboratory would encourage all sentinel laboratories to obtain travel history as well, as a safety precaution.

If a patient meets the criteria for a suspect Influenza A (H5N1) case (see below) or if you have any questions or concerns relating to H5N1, please contact the Epidemiology Division at 863-7240 or 1-800-640-4374 (VT only). These phone numbers are also available after normal business hours and weekends/holidays.

The VDH Laboratory does not recommend that providers perform a rapid Influenza assay on specimens from patients with suspected H5 infection unless avian influenza has been established in the region and a biological safety cabinet is available. Clinical respiratory specimens from patients meeting CDC's clinical and epidemiological criteria should be sent to the VDH Laboratory, where they will be analyzed by PCR for avian influenza. Serum and autopsy specimens may be sent through the VDH Laboratory to CDC for analysis.

Arrangements for a courier dispatch and testing will be established once the Epidemiology Division has been informed.

Influenza A(H5N1) Virus Infection Criteria

Testing for influenza A(H5N1) is indicated for **hospitalized** patients with:

- a. radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternate diagnosis has not been established, **AND**
- b. history of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza in poultry and/or humans (for a listing of H5N1-affected countries, see CDC's website at <http://www.cdc.gov/flu/pandemic/> or the OIE Web site at http://www.oie.int/eng/en_index.htm and the WHO Web site at <http://www.who.int/en/>).

Testing for influenza A(H5N1) should be considered on a case-by-case basis in consultation with state and local health departments for **hospitalized or ambulatory** patients with:

- a. documented temperature of >38°C (>100.4°F), **AND**
- b. one or more of the following: cough, sore throat, shortness of breath, **AND**
- c. history of contact with domestic poultry (e.g., visited a poultry farm, household raising poultry, or bird market) or a known or suspected human case of influenza A(H5N1) in an H5N1-affected country within 10 days of symptom onset.

Specimens for Collection

Respiratory specimens

- a. nasopharyngeal or oropharyngeal aspirates or washes in viral transport medium
- b. nasopharyngeal or oropharyngeal swabs in viral transport medium
- c. sputum
- d. bronchoalveolar lavage
- e. tracheal aspirates

Swab specimens should be collected only on swabs with a Dacron tip and an aluminum or plastic shaft. Calcium alginate and cotton tipped swabs and swabs with wooden shafts are unacceptable.

Specimens should be kept at refrigerated temperature (4°C)

Serum specimens

Acute-phase serum should be collected soon after onset of clinical symptoms and no later than 7 days after onset of symptoms and stored frozen. The convalescent-phase serum should be collected 14 days after onset of symptoms.

Both acute and convalescent specimens should be sent to the VDH Laboratory for forwarding to the CDC for serological testing. Sera may be kept at 4°C for up to 7 days, but afterwards must be kept frozen at -20°C.

Autopsy Specimens

CDC can perform immunohistochemical (IHC) staining. A minimum total of 8 blocks or fixed tissue specimens representing samples from each of the following sites should be obtained:

- a. Central (hilar) lung with segmental bronchi
- b. Right and left primary bronchi
- c. Trachea (proximal and distal)
- d. Representative pulmonary parenchyma from right and left lung

In addition, representative tissues from major organs should be submitted for evaluation. For patients with suspected myocarditis or encephalitis, include myocardium (right and left ventricle) and CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum). Tissues from any other organs showing significant gross or microscopic pathology should also be included.

Submit these specimens as:

- a. Fixed, unprocessed tissue in 10% neutral buffered formalin, or
- b. Tissue blocks containing formalin-fixed, paraffin-embedded specimens, or
- c. Unstained sections cut at 3 microns placed on charged glass slides (10 slides per specimen)

Please include a copy of the autopsy report (preliminary or final) and a cover letter outlining a brief clinical history. Please also include the name, title, mailing address, phone and fax numbers of the submitter in the event that CDC pathologists require further information.

Specimens should be sent to the VDH Laboratory at room temperature (NOT FROZEN).